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TITLE: Vascular Plaque Determination for Stroke Risk Assessment

PRINCIPAL INVESTIGATOR: Vince, David Geoffrey

CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation
Cleveland, OH 44195-0001

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14. ABSTRACT
Each year, about 800,000 Americans experience a new or recurrent stroke. The long-term goal of this research program is to create non-invasive methods utilizing ultrasound to identify plaques at high risk for initiating a cerebrovascular accident. The core of the current research project is a pilot clinical study to enroll 100 subjects who are scheduled for carotid endarterectomy (CEA. From each subject, the research effort obtains ultrasound data from the carotid plaque (or carotid artery for Normal subjects) prior to surgery and then creates histology slides of the removed plaque tissue which are used to train a statistical classifier for determining plaque composition. The main accomplishment from the prior year is the enrollment of 32 CEA subjects and thus exceeding the projected target of 25 subjects in Year 1. In addition, the normal subject enrollment and data collection was completed. The normal subject data is important for signal processing of the backscattered signals from carotid plaque.

15. SUBJECT TERMS
Atherosclerosis, stroke, cerebrovascular accident, carotid endarterectomy, ultrasound, spectral analysis, tissue characterization, machine learning, noninvasive, carotid plaque

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TABLE OF CONTENTS

1. INTRODUCTION

Each year, about 800,000 Americans experience a new or recurrent stroke with a disproportionate number of these being veterans. Over 15,000 veterans are hospitalized each year for cerebrovascular accidents including stroke. The long-term goal of this research program is to create **non-invasive** methods utilizing ultrasound to identify plaques at high risk for initiating a cerebrovascular accident. As a critical first step toward achieving this long-term goal, the objective of this research project is to test the hypothesis that non-invasive spectral analysis of diagnostic ultrasound backscatter from carotid plaque can accurately and reproducibly determine plaque composition. The core of this research project is a pilot clinical study to enroll 100 subjects who are scheduled for carotid endarterectomy (CEA). From each subject, the research effort obtains ultrasound data from the carotid plaque prior to surgery and then creates histology slides of the removed plaque tissue which are used to train a statistical classifier for determining plaque composition. Future studies will apply this tool to the task of predicting stroke in "at risk" populations.

2. KEYWORDS:

Atherosclerosis, stroke, cerebrovascular accident, carotid endarterectomy, ultrasound, spectral analysis, tissue characterization, machine learning, noninvasive, carotid plaque

3. ACCOMPLISHMENTS:

This research project, *Vascular Plaque Determination for Stroke Risk Assessment*, contains five major tasks in order to create the non-invasive tool for determining plaque composition.

Major Task 1: *Clinical Study Preparation*
This effort covered the items necessary to begin clinical enrollment for the Ultrasonic Mapping of Carotid Plaque Composition Study (UMP Study). The statement of work proposed completion of these tasks by the second month (i.e. 14 Nov 2016) of the effort. These items with their completion dates are as follows:

- Development of the ultrasound standard operation procedures
  - ***Completed*** prior to contract effective date of 15 Sep 2016
- Creation of required clinical documentation (Clinical Trial Folder, Case Report Forms, etc.)
  - ***Completed*** prior to contract effective date of 15 Sep 2016

The first two *milestones* of the project were the human studies approvals required to begin recruitment. The original planned completion dates are in parentheses:

- Local Institutional Review Board Approval: 23 Aug 2016 (14 Nov 2016)
- HRPO Approval: 31 Aug 2016 (14 Jan 2016)

Major task 1 was completed prior to the effective date of the contract**.**

Major Task 2: *Subject Enrollment and Data Acquisition*
The enrollment target for this effort is 100 subjects following the contract effective date. The enrollment and data collection is ongoing with a targeted completion by 15 Jun 2019. Actual enrollment in Year 1 was 32 subjects which **exceeded** the projected enrollment of 25 subjects.

The subtasks and first year status are as follows:

- Consent Subjects (**goal 25%**): 32% complete
- Research Ultrasound Imaging: (**goal 25%**): 32% complete
- Collection of Plaque from Carotid Endarterectomy (**goal 25%**): 31% complete

Target completion of enrollment milestone remains 14 June 2019.

Major Task 3: Data Processing

These tasks are ongoing for processing of the plaque tissue and ultrasound data. Tasks were begun 4 November 2016 and are expected to continue through July 2019. These tasks include the following:

- Prepare Serial Histology of Carotid Plaque 25% complete
- Ultrasound Signal Processing Development and Testing began Mar 2016
- Match Slides to Ultrasound Grayscale *In Vivo* Data set to begin in Oct 2017

The Task 3 milestone is extraction of the ultrasound spectral parameters from all regions of interest (ROI) with matched histology. Expected completion is August 2019.

Major Task 4: Create Statistical Classifiers

Planned start of Mar 2018 remains unchanged.

Initial statistical classifier creation is planned for Sep 2018.

Milestone: Final statistical classifiers - target Aug 2019.

Major Task 5: Validation of Statistical Classifiers

Planned start Aug 2019 remains unchanged.

Milestone: Validated statistical classifiers – target Sep 2019.

What was accomplished under these goals?

1. Launching of the clinical study and enrollment

Prior to the contract effective date all tasks were completed for Major Task 1, *Clinical Study Preparation*. This left the task of finalizing the recruitment process. Dr. Sean Lyden, co-investigator for this effort, led the effort to include vascular surgeons in subject recruitment efforts. Currently, all vascular surgeons that perform carotid endarterectomies (CEA) at the main campus of the Cleveland Clinic are involved in patient recruitment. Thus all patients scheduled for a CEA are evaluated for the clinical study, *Ultrasonic Mapping of Carotid Plaque Composition* (UMP). At the end of the first year's effort, the following surgeons are assisting with patient recruitment: Behzad Farivar, Rebecca Kelso, Lee Kirksey, Sean Lyden, Federico Parodi, Christopher Smolock, and Sunita Srivastava.

The first subject was enrolled 4 November 2017. Table 1 contains an enrollment breakdown by quarter compared with the projected recruitment as stated in the grant proposal. As depicted in the Table 1, the study enrollment of 32 subjects is ahead of the projected goal for Year 1 of 25 subjects.

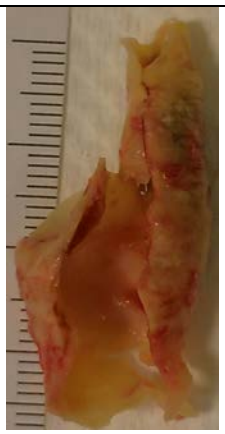
Table 1: Projected versus actual enrollment for the UMP Study. The actual enrollment for the first year of 32 exceeded the projected first year enrollment of 25.

	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Projected	*0*	*7*	*9*	*9*
Actual	**4**	**7**	**11**	**10**
Actual Cumulative	4	11	22	32

2. Carotid Plaque Tissue Processing

A study representative collects the tissue following excision of the plaque during the CEA procedure. The plaque is placed in saline and RF ultrasound data is collected using the same settings as used during the in vivo imaging prior to the CEA. A Siemens Acuson S3000 system using 9L4 transducer is used for the data acquisition. This system has Axius Direct software that permits RF data acquisition. The in vivo data collection obtains ultrasound RF data from sites that are spaced roughly every 1 cm from the proximal to the distal end of the plaque. The primary reason for the ex vivo data collection is to aid in the matching of the in vivo transverse sites with histology slides from the plaque. An important dataset in locating these same sites between the ex vivo and in vivo data are systematic RF data collections while moving the transducer from proximal end to the distal end of the transducer. These images obtained from sweeping through the plaque greatly aid the site matching since they depict how the plaque is changing in both proximal and distal directions.

UMP0044
(0.5 cm scale)



UMP0051
(mm scale)

UMP0060
(mm scale)

UMP0070
(mm scale)

Figure 1: Photographs of example excised tissue following removal during CEA. All samples collected during the first year of this project.

Following ex vivo ultrasound data collection, the tissue is placed in Formalin and then in Cal-Rite in order to decalcify the plaque. Prior to paraffin embedding, the in vivo sites are located again using the ultrasound system and marked using ink. The inked region is visible when cutting the paraffin embedded tissue and aids in the final matching step between the in vivo site and histology.

As of 14 September, 31 plaque samples were collected and processed with histology slides produced from 25 of the plaque specimens. Figure 1 depicts some of the plaque specimens gathered under this research effort.

3. Completion of the Normal Subject Enrollment

A part of this clinical study involves the recruitment of normal subjects. One of the approaches for determining attenuation compensation is based on data from normal subjects. For this study, normal is defined as subjects who have not had nor are scheduled for an intervention involving the carotid artery. For each normal subject carotid artery, five sites are insonified and backscattered RF data is collected using the same settings used with the CEA group. Similar to the CEA group of subjects, these sites are spaced by 1 cm and centered on the bifurcation of the internal and external carotid arteries. Thus for the six enrolled subjects a total of 60 independent sites were obtained.

One of the approaches proposed to compensate for the effects of attenuation is to use adventitia from normal subjects. To facilitate this regions of interest (ROI) within the adventitia are drawn in the grayscale images and the corresponding RF ultrasound data is extracted (see Figure 2). A total of 191 adventitial sites have been located from the normal subject data. Each ROI is 64 points long by 15 lines which is approximately 1.2 mm by 1.2 mm. The first step in the processing chain is to estimate the average power spectrum for the ROI. A Yule-Walker auto-regressive approach is applied to estimate the spectral power for each line and then the log of the power is averaged together (i.e. dB). Averaging in the log domain minimizes the effects of cuts in the power spectrum arising from phase interference, speckle variation, etc.

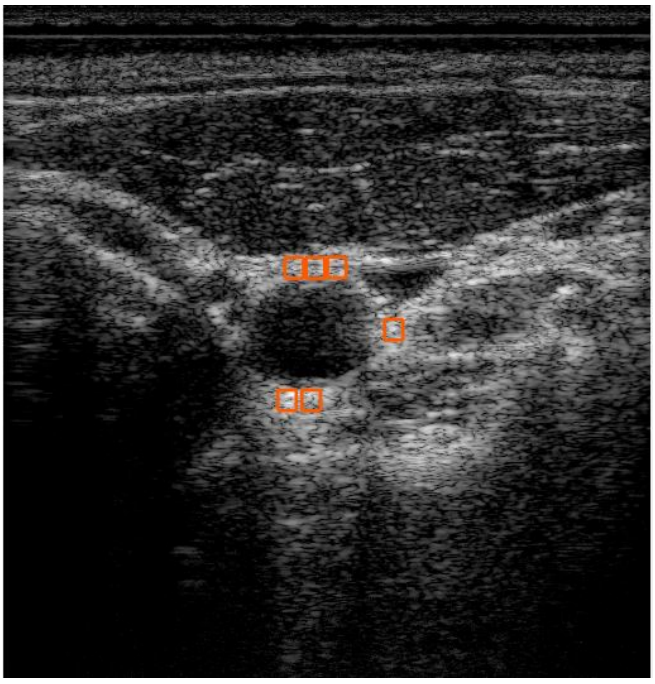


Figure 2 ROI placement within adventitial regions for normal subjects. Image is 4 cm deep with a 2 cm transmit focus.

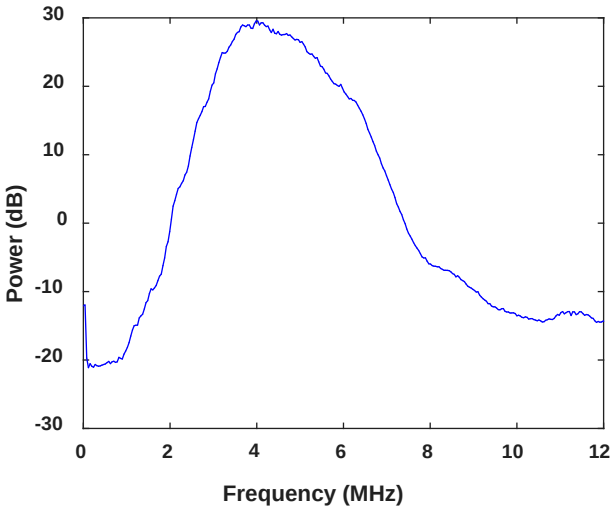


Figure 3 Power spectrum obtained from reference phantom (CIRS model 044) using Siemens Acuson S3000 ultrasound system with a 9L4 transducer.

The ROI power spectrum estimate is normalized by a reference spectrum obtained from a CIRS Model 044 phantom. The reference power spectrum is obtained by collecting RF data with the same settings as the *in vivo* data collection. The target in the phantom is a region within the 0.5 dB/cm-MHz side with no inclusions and the transducer is moved to sample different areas within this uniform region of the phantom. The reference spectrum is computed by first estimating the power for each line within the frame at the same depth position as the tissue ROI. These spectral estimates are averaged together for all lines within the frame and over multiple frames in order to obtain a relatively clean power spectrum that incorporates the effects of diffraction, transmit and receive transfer functions for the ultrasound system and transducer, and to partially compensate for attenuation. After subtracting the ROI power spectrum using the reference power spectrum in dB, the remaining effects of attenuation of the overlying tissue remains to be addressed. A representation of the reference power spectrum is shown in Figure 3. The bandwidth of the system is obtained from the reference spectrum:

- 3dB Bandwidth: 3.4 MHz to 5.0 MHz
- 20dB Bandwidth: 2.5 MHz to 6.8 MH

These bandwidths will be used when creating statistical classifiers in order to limit the frequency domain from which spectral parameters are extracted.

Stated Goals Not Met

Due to the focus on UMP study recruitment and the delay in hiring the research engineer, segmentation of the carotid plaque prior to paraffin embedding was delayed. As of 14 September 2017, the backlog stands at 5 samples which we plan to process during Quarter 1 of Year 2.

What opportunities for training and professional development has the project provided?

IEEE International Ultrasonics Symposium
Washington D.C., USA
6-9 September 2017
Attended by: Russell J. Fedewa (Co-investigator) and Sheronica James (Research Engineer).

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

Continue Study Enrollment

This research effort depends on continuing enrollment of subjects into the study. We expect to meet or exceed the projected goal of a cumulative total of 67 subjects enrolled by the end of Year 2 (see Table 1).

Attenuation Compensation Comparison and Investigation
Completion of an evaluation of attenuation compensation approaches to determine which approach is best for differentiating carotid plaque tissue types. The following approaches will be evaluated:

1. Reference phantom only based compensation
2. Adventitia based approaches, where data from normal subjects is used to provide an estimate of the average attenuation experienced by the ultrasound waves.
3. Using the ex vivo data as a generalized reference to obtain an average attenuation compensation approach
4. Centroid methods including:
   K. Samimi, T. Varghese, Optimum Diffraction-Corrected Frequency-Shift Estimator of the Ultrasonic Attenuation Coefficient, IEEE Trans. UFFC, 2016, vol 63 (5), 691-702.

Produce First Statistical Classifier
This involves completion of the following tasks:

1. Determine histology slide match to each in vivo site for the first 32 subjects
2. Locate homogenous regions within each of these matched histology slides sites and define corresponding ROI's in the RF ultrasound data.
3. Extract spectral parameters from the ROI's. Spectral parameters include: integrated backscatter, linear fit parameters (mid-band fit, intercept, and slope), maximum and minimum values and the associated frequencies.
4. Use the results from step 3 and the tissue type information from step 2 to produce a Random Forest statistical classifier for two key functions:
   a. Determine which parameters are most important for classification
   b. Use classifier to determine the best signal processing options to use in the final classifier production

The information obtained from these three tasks will form the foundation for the final statistical classifier creation and validation in Year 3.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

It took five months to recruit a suitably qualified Research Engineer. This has delayed progress on signal processing efforts, but these delays are not expected to impact hitting milestones for the project.

The project is currently exceeding the enrollment projections due in part to starting enrollment earlier than anticipated and exceeding the projected recruitment for two additional quarters. However, we anticipate that we will not meet the projected enrollment targets consistently for Quarter 3, Year 2 through Quarter 2, Year 3. These four quarters have a projected enrollment of 12 subjects per quarter. Based on the limited experience in Year 1, we do not anticipate consistently exceeding 10 subjects per quarter. Thankfully, we are ahead of enrollment target at the end of Year 1 by 7 subjects which is very close to the anticipated gap of 8 subjects and thus the enrollment completion target number and time remain unchanged.

Changes that had a significant impact on expenditures

It took five months to recruit a suitably qualified Research Engineer for the project with the corresponding reduced spending on salary for the engineer position.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No significant changes in the use or care of human subjects.

Annual IRB Review and Approval - 11 August 2017
IRB Study Number 12-797
"Ultrasonic Mapping of Carotid Plaque Composition (UMP)"
PI: David Geoffrey Vince, PhD
Cleveland Clinic Foundation, Cleveland, OH
HRPO Log Number A-19566

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

As provided in the Data and Resource Sharing Plan, the collected ultrasound RF backscatter data along with Matlab processing code will be uploaded to the Zenodo digital research sharing site by 1 Aug 2020.

Currently, in vivo data from 32 human carotid plaques and the surrounding tissue has been collected and archived internally in the Lerner Research Institute at the Cleveland Clinic. This data comprises the following:

- Reference Phantom Data for Normalizing the collected RF data
- 114 Static Sites:
  - in vivo RF ultrasound backscattered signal from human carotid plaque with a 1 cm separation to insure independence.
  - 10 frames of data per file
    - Each Frame: 456 lines by 2076 points at 40 MSamples/sec.
    - Collected using pulse inversion
- 32 Sets of Transverse and Longitudinal Scans (Same settings as the Static Sites):
  - Transverse Scans Through the Plaque: Sonographer slowly moving the transducer from proximal to the distal end of the plaque while collecting RF data.
  - Longitudinal Scans Through the Plaque: Sonographer slowly moving the transducer from medial to lateral while collecting RF data from the plaque.
- Data from 6 Normal Subjects (Similar to RF ultrasound data from CEA subjects)
  - 60 static sites
  - 6 sets of transverse and longitudinal scans

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	D. Geoffrey Vince
Project Role:	Principal Investigator
Research Identifier (e.g. ORCID ID):	0000-0001-6155-7118
Nearest Person Month Worked:	3
Contribution to Project:	Project management including all submissions, manuscripts, and communications. Oversight and coordination between departments involved in the research effort: Biomedical Engineering, Vascular Surgery, and Vascular Medicine. Project management and communications. Management of histology processing and interpretation. Review, guidance, and development of signal and data processing approaches.
Funding Support:	NIH/NHLBI The Cleveland Clinic Innovation Accelerator

Name:	Russell J. Fedewa
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID):	0000-0002-0690-9472
Nearest Person Month Worked:	12
Contribution to Project:	Directing the development of all signal processing approaches. Study coordinator duties. Data collection and processing. Development of clinical study protocol, procedures, and forms with assistance from co-investigators and principal investigator.
Funding Support:	NA

Name:	Sheronica James
Project Role:	Research Engineer
Research Identifier (e.g. ORCID ID):	0000-0002-5647-1106
Nearest Person Month Worked:	7
Contribution to Project:	Signal processing development to account for effects of attenuation and diffraction. Data collection and processing. Support the implementation and execution of subject recruitment and enrollment, specifically, the consent process.
Funding Support:	NA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Name/Role: D. Geoffrey Vince / Co-investigator
Description of Change: **Grant closed**
Title: ARFI [Acoustic Radiation Force Impulse] and B-Mode Ultrasound Imaging for Transverse Carpal Ligament Pathomechanics
Sponsor: NIH NIAMS, 1R21AR064957-01A1

Name/Role: Russell J. Fedewa / Principal Investigator
Description of Change: **Grant closed**
Title: Ultrasonic Determination of Human Carotid Plaque Composition
Sponsor: American Heart Association, 15SDG25700258

Name/Role: Sean P. Lyden / Site Principal Investigator
Description of Change: **Study Site Closed**
Title: Carotid Revascularization and Medical Management for Asymptomatic Carotid Stenosis Trial (CREST-2) (PI – Brott)
Sponsor: NIH NINDS, 5U01 NS080168

What other organizations were involved as partners?

Organization Name:	Siemens Medical Solutions USA, Inc.
Location of Organization:	51 Valley Stream Parkway, Malvern PA 19355, USA
Partner's Contribution to the Project:	In-kind support
	Equipment Loan: S3000 HELX Ultrasound System
	9L4 Transducer
	Axius Direct Research Interface
	Matlab Software to Access RF Data Files

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART: See Appendix

9. APPENDIX:

- **Quad Chart**

Vascular Plaque Determination for Stroke Risk Assessment

PR151297
W81XWH-16-1-0608

PI: Vince, David Geoffrey **Org:** Cleveland Clinic Foundation, The **Award Amount:** $1,756,026

Study Aims

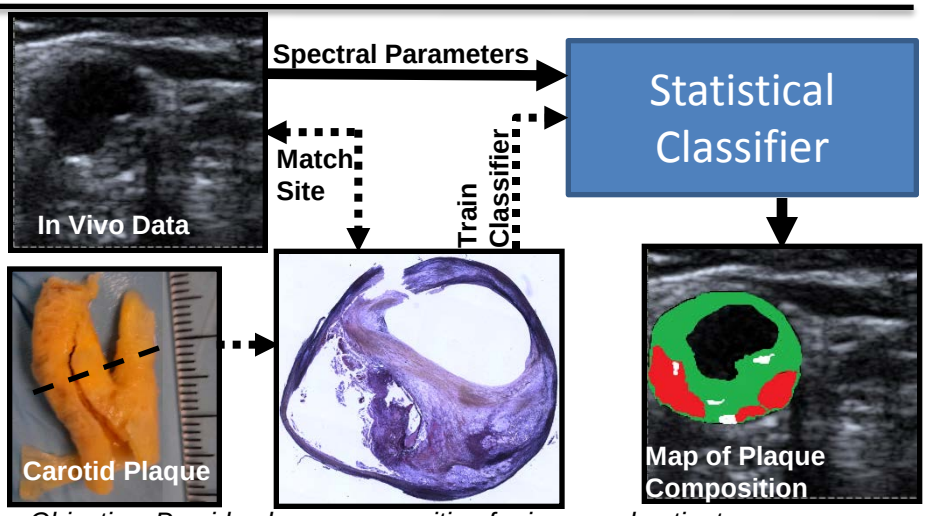
- Obtain in vivo ultrasonic backscatter from human carotid plaque.
- Correlate regions in the ultrasound images to histology of the human carotid plaque.
- Create a set of statistical classifiers for non-invasive determination of carotid plaque composition using the ultrasound spectral parameters as input values.
- Validate and test the statistical classifiers

Approach

Clinical study of 100 patients scheduled for carotid endarterectomy (CEA) to remove clinically significant plaque. Key data:

- Ultrasound RF signals backscattered from plaque prior to CEA
- Serial Histology of excised carotid plaque following CEA

Spectral parameters from the RF data are used as input values for a statistical classifier where the output is plaque composition. The classifier is trained using the matched histology slides.



Objective: Provide plaque composition for improved patient care.
Accomplishment: Enrolled 32 of 100 CEA subjects exceeding CY17 target of 23. Finished enrollment for normal subjects.

Timeline and Cost

Activities CY	17	18	19
Clinical Study Preparation			
Subject Enrollment and Data Acquisition			
Data Processing			
Create Statistical Classifiers			
Validation of Classifiers			
Estimated Budget ($K)	**$553**	**$608**	**$594**

Updated: 13 Oct 2017

Goals/Milestones

CY17 Goal – Begin Clinical Study and Data Processing
☑ Local IRB Approval
☑ HRPO/ACURO Approval
CY18 Goal – Initial Classifier Construction
☐ Create first statistical classifier based on initial data
CY19 Goal – Complete Enrollment and Validate Classifiers
☐ Complete enrollment
☐ Correlate regions in the ultrasound images to histology
☐ Statistical Classifiers Finished
☐ Validation of Classifiers

Comments/Challenges/Issues/Concerns
The primary cause of the discrepancy between the projected and actual expenditures is delayed hiring of a Research Engineer.

Budget Expenditure to Date
Projected Expenditure: $553,328.00
Actual Expenditure: $406,528.81